PLATELETS IN CHRONIC SMOKERS SHOW A HYPERACTIVE RESPONSE IN VITRO TO A FOREIGN SURFACE

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INTRODUCTION

A number of investigators have evaluated platelet function in smokers. The results have been diverse. Part of the lack of clarity may relate to the diversity of the characteristics of platelet function that were evaluated, as well as the length of time following smoking during which the test was performed. In this chapter, we will review the literature in this regard, with particular emphasis upon the more prolonged effects of smoking. In particular, we will review an investigation of the effects upon platelets of chronic smoking that we previously performed (1).

METHODS

We studied the effect of cigarette smoking upon platelets in 23 individuals who had never smoked (nonsmokers); and 20 persons who had smoked various quantities of nicotine-containing cigarettes (smokers) (1). None of the subjects had taken any antiplatelet or anticoagulant drugs (aspirin, dipyridamole, allopurinol, sulfinpyrazone, propranolol, heparin, coumadin) for at least one month prior to the investigation. None of the smokers had smoked a cigarette for at least 4 hours prior to any platelet studies. Among the smokers there were 10 men and 10 women. Among the non-smokers there were 15 men and 8 women. The levels of cholesterol, uric acid, triglycerides, glucose, and urea nitrogen (BUN) were similar in both groups. The daily consumption of cigarettes among smokers was $1.4 \pm 0.5$ packs, and the duration of smoking was $19 \pm 12$ years. Platelet function was measured both in the early phase of activation (adhesion, formation of pseudopodia and spreading) and in the later phases (aggregation as well as the release of platelet-specific proteins) (1).

PLATELET ELECTRON MICROSCOPIC SURVEY

The platelet electron microscopic survey that we performed will be described in somewhat more detail than the other investigations of platelets, because the technique is less well known, although it has been described in detail previously (1-3).
The procedure is a standardized in vitro method that provides a morphologic assessment of three separate phases of the platelet response (adhesion, surface activation and aggregation). The major advantages of this method, are that one can directly observe structural changes after platelets contact an activating surface in vitro, and the test monitors the early phase of the platelet response (Fig 1).

A 2 ml whole blood sample was withdrawn and discarded to eliminate any procoagulant substances that might have been introduced during venipuncture. Nine ml of whole blood was withdrawn into the 3.8% sodium citrate, which was mixed briefly with the whole blood. The anticoagulated blood specimen was transferred immediately to a 6 oz polyethylene, square bottle with a wide mouth and a nonfrosted microscope slide covered with a thin film of Formvar (1% Formvar dissolved in ethylene dichloride) was introduced horizontally. The blood mixture remained in contact with the Formvar film for exactly eight minutes. During this incubation, blood platelets became attached to the surface of the Formvar film. Afterward, the microscope slide was removed from the bottle, drained briefly, and rinsed in Tyrode's solution. Rinsing continued until erythrocytes were no longer grossly visible on the film surface. The slide was then immersed in cacodylate buffered 3% glutaraldehyde for 15 minutes at room temperature to stabilize the structure of the adherent platelets. After fixation, the slide was rinsed and air dried.

The edges of the slide were scraped with a scalpel blade to free the Formvar film. Stainless steel, 200 mesh specimen grids were placed over representative areas so that their convex surfaces contacted the Formvar film. After mounting, platelet populations were viewed with a transmission electron microscope operated at 50 KV.

In this procedure, the number of platelets present on the surface of the Formvar film (a polyvinyl formal plastic film) provided a measure of their adhesive capacity. Surface activation of single platelets was demonstrated by the extrusion of pseudopodia and cytoplasmic spreading between pseudopodia with reorganization of internal organelles. Aggregation, indicative of the cohesive capacity of each population of